

# ABO AND RHESUS BLOOD GROUP PREVALENCE IN DISTRICT PESHAWAR, PAKISTAN, A CROSS-SECTIONAL STUDY

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## ABSTRACT

**BACKGROUND:** This research study was planned to determine ABO and Rhesus blood group prevalence in the population of Peshawar who sought services in the hematology laboratory of Hayatabad Medical Complex, Peshawar, Pakistan.

**METHODOLOGY:** The cross-sectional study, was conducted from August 10 to December 25, 2023, at HMC in Peshawar after ethical board approval. A total of 5342 participants were determined using convenient sampling. Participants of both genders over four months were included, excluding those under four months and unwilling. Informed consent was obtained, and demographic data were collected via a dedicated questionnaire. Blood samples, collected aseptically, underwent blood group determination using the Forward and Reverse tube method, with data analysis conducted using SPSS V26

**RESULTS:** Out of a total of 5342 participants, the most common ABO was B at 37.01% (1974), then O at 27% (1462), A at 26% (1410), and AB at 10% (496). The majority, 94% (2512), had Rh-positive blood types, with 6% (314) being Rh-negative. Among males, the most common ABO blood group was B at 39.62% (1058), followed by O at 30.13% (804) and A at 22.09% (590), while AB was the least common at 8.16% (218). In females, B was the most common at 34.28% (916), followed by A at 30.68% (820), O at 24.62% (658), and AB at 10.42% (278).

**CONCLUSION:** The study found B as the most common ABO blood group, AB the least, consistent across genders. Rh-positive types were 94%, with females having a higher Rh-negative prevalence. B+ve was common among Rh+ve, and A-ve among Rh-ve individuals.

**Keywords:** ABO, Blood group, Tube method,

## INTRODUCTION

In 1901, a Viennese physician and pathologist, Karl Landsteiner, made a significant breakthrough by discovering a system of blood groups known as ABO(1). This system classifies blood into four main groups—O, A, B, and AB—based on specific carbohydrate sugars on the membrane of RBC (red blood cell). The A antigen is characterized by N-acetylgalactosamine, and the B antigen by D-galactose(2). Type O blood results when the H antigen remains unaltered, preventing the attachment of either the A or B antigens to red blood cells(2). Reuben Ottenberg initiated the practical application of blood typing in 1907, notably during the First World War, and it was extensively used (1914–1915).

The multiple alleles of the ABO blood groups are situated on the long arm of the ninth chromosome (9q34), displaying a Mendelian inheritance pattern(3).

The Rhesus (Rh) blood group system, founded in 1941, is based on specific inherited antigenic proteins on the surface RBC(4). Among the 29 known human blood group systems, the ABO and Rh system stands out as one of the most significant in clinical terms (4). The Rhesus (Rh) blood group system includes 50 different types of antigens. It primarily recognizes the five most critical antigens: "D, C, c, E, and e", which are part of the Rhesus proteins present on the membranes of RBC(red blood cell) (5).

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Determining blood type involves identifying antigens on the RBC surface through the forward method and detecting specific isoagglutinin in plasma using the reverse method (World Health Organization, 2011)(6). Methods based on antibodies have been the standard for the detection of ABO, Rhesus, and "extended" blood group antigens on the membrane of RBC since the ABO system was discovered (7). A blood group antigen is a protein found on red blood cells that differs

between individuals and can incite the production of immune antibodies when encountered, such as during pregnancy or blood transfusion(7). When blood comes into contact with an antibody not typically present in it, agglutination occurs. For instance, Blood A type contains the isoagglutinin called Anti-B, Blood B has Anti-A, while blood type O lacks antigens but contains both isoagglutinin (6). For all donors and recipients, both "forward (cell) and reverse (serum) grouping tests" are necessary to determine the ABO group (8). Tube method, the gold standard technique, is used to determine ABO and Rhesus blood group (Rh) (9)

In one research study in Islamabad, Pakistan, B+ (31.2%) was the most frequent blood group, while AB- was the lowest (0.6%). The prevalence of others was as follows: "A+ (21.5%), AB+ (9.8%), O+ (29.7%), A- (1.8%), B- (2.9%), and O- (2.5%)". The percentages of Rh+ and Rh- blood groups were "92.2% and 7.8%" (10). In Srinagar, Uttarakhand, India, B (31.68%) was the most common, while AB was the lowest (11.70%). Rhesus positive and negative distribution was 93.51% and 6.49%, respectively(11). In Switzerland, the proportions of blood groups were as follows: "A (47.2%), B (8.4%), and AB (3.0%)" (12). In a study conducted in the western region of Saudi Arabia, results indicated that blood group O had the highest proportions at 50.1%, then "A (29.7%), B (16%), and AB (4.1%)". The prevalence of Rh-positive was 91.3%, and Rh-negative was 8.63%

This research study was planned to determine ABO and Rhesus (Rh) blood group distribution in the population of Peshawar who come upon the hematology laboratory of HMC (Hayatabad Medical Complex), Peshawar, Pakistan.

## **METHODOLOGY**

This study was a descriptive cross-sectional which was performed in the haematology laboratory of Hayatabad Medical Complex Peshawar, spanning three months, starting from August 10, to December, 25 2023. The sample size was calculated using the Open Epi Calculator to be 5342. A convenient sampling technique was adopted to recruit individuals in this research study. Both genders, age groups greater than four months, and those willing were included, but those less than four years of age and unwilling were also excluded from the study.

## **CONSENT**

After approval from the ethical and advanced research board, a purposefully built

questionnaire including all the demographic information and a consent form explaining research objectives and methodology was developed. Informed written consent was taken from all recruited willing participants, and all the demographic information was then obtained on the purposefully built questionnaire.

## **BLOOD SPECIMEN COLLECTION**

All the recruited participants were then asked for blood sample collection. A qualified phlebotomist using aseptic techniques took blood samples from all participants. Blood samples (3-5ml) were collected in EDTA and GEL tubes. Gel tubes contain clot activators and are used for serum separation, while EDTA tube contains anticoagulants to prevent clotting. Blood specimens collected in EDTA and GEL tubes were then used for blood group determination by Tube methods.

## **TUBE METHOD**

Blood samples in EDTA tubes were used for forward blood grouping, and blood samples in GEL tubes were used for reverse blood grouping. The forward grouping suggests the presence or absence of A and B antigens on the surface of RBCs, whereas reverse grouping indicates the presence or absence of anti-A and anti-B in serum(14).

## **FORWARD GROUPING (TUBE METHOD)**

The forward grouping for ABO determination was conducted using the tube method. A 3-5% suspension of RBCs in isotonic saline (prepared from washed cells) was employed. In two labelled test tubes, one drop of Anti-A sera and one drop of Anti-B sera were added separately. A drop of the RBC suspension was then added to each tube and mixed thoroughly. Subsequently, the tubes were centrifuged for 15 seconds. The cells were gently mixed and inspected for agglutination both macroscopically and microscopically, with the results being duly recorded(14).

## **REVERSE BLOOD GROUPING FOR ABO BLOOD GROUP (TUBE METHOD)**

The reverse blood grouping for the ABO blood group utilized the tube method. Two labelled test tubes, one for A1 and another for B, were set up for each known RBC. Two drops of the serum/plasma to be tested were added to each respective labelled tube. Following this, one drop of known A1 and B cells was introduced into the tubes and centrifuged for 15 seconds. The cells were gently mixed and examined for agglutination both macroscopically and microscopically, and the

outcomes were duly recorded. Additionally, a table was employed to interpret the results

based on the reactions observed(14).

**TABLE 1: THE FORWARD GROUPING FOR THE RH BLOOD GROUP (TUBE METHOD)**

Forward		Reverse		Result
“Anti-A”, “Anti-B”		“A1 cells”	“B cells”	
“Positive”	“negative”	“Negative “	“ positive”	A
“Negative:	“positive”	“Positive”	“negative”	B
“Negative”	“ negative”	“Positive”	“ positive”	O
“Positive”	“positive”	“negative”	“negative”	AB

The Rh blood grouping, specifically testing for the Rh D factor, utilized reagent Anti-D and the patient's RBCs. The RBCs of the patient were washed multiple times with a saline solution (isotonic), mixed with anti-Rh-D, and centrifuged for 15 seconds. Agglutination indicated the presence of the D antigen (Rh D factor), and this reaction was observed both macroscopically and microscopically to determine if the patient was Rh D positive. Conversely, the absence of agglutination labels the participant as Rh negative.

All the data was recorded, and statistical analysis was performed using SPSS v26. P values less than 0.05 were considered statistically significant.

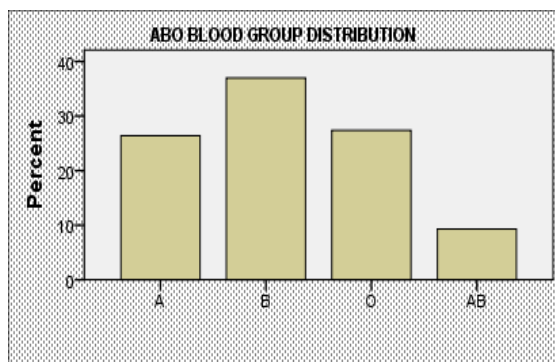
## RESULTS

### GENDER-BASED PREVALENCE

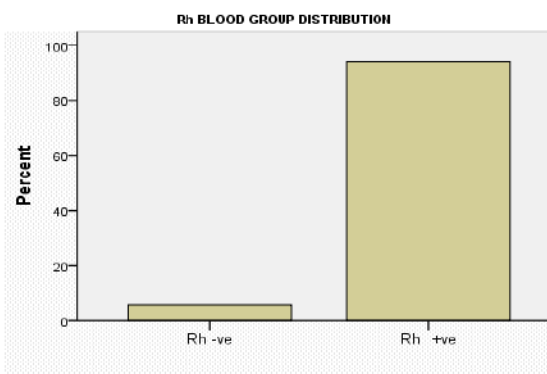
The total participants in this study were 5342. Males were 50% (2670), while females were 50% (2672). 26.40% (1410) of the participants were having blood group A, 37 % ( 1974) of the participants were having blood group B, 1462 (27%) of the participants were having blood group O while 496 (9.3%) of the participants were having blood group AB. The highest number of blood groups in our participant was B, and the lowest was AB in the ABO blood group. 94% (2512) of the participants were Rh-positive, while 6% (314) were Rh-negative, as shown in Table 01 and Figures 01 and 02.

**TABLE 2: GENDER-BASED PREVALENCE**

	FREQUENCY	PROPORTION	TOTAL
<b>GENDER</b>			
MALE	2670	50%	5342
FEMALE	2672	50%	5342
<b>ABO BLOOD GROUP</b>			
A	1410	26%	5342
B	1974	37%	
O	1462	27%	
AB	296	10%	
<b>RH BLOOD GROUP</b>			
RH +ve	5032	94%	5342
RH-ve	314	6%	5342



**Figure 01: ABO Blood Group Distribution**



**Figure 02: Rh Blood Group Distribution**

### GENDER WISE DISTRIBUTION

The B 39.62% (1058) was the most commonly found ABO blood group in males, then O 30.13% (804) and A 22.09% (590), while AB 8.16% (218) was the lowest ABO blood group in males. Similarly, B 34.28% (916) was the high-frequency ABO blood group in females, then A 30.68% (820) and O 24.62% (658), while AB 10.42% (278), the lowest ABO blood group in females as shown in table 02. The association of gender with the ABO blood group was statistically significant ( $p < 0.001$ ). Rh-positive 95.80% (2558) was the highest frequency Rh blood group in males, while the Rh-negative blood group in males was 4.20% (112). Similarly, the most common Rh blood group in females was Rh positive 92.44% (2470), while the Rh negative blood group was 7.56% (202), as shown in Table 02. The gender association with the Rh blood group was statistically significant with a p-value  $< 0.000$ .

**TABLE: 3 GENDER WISE DISTRIBUTION**

	Males (%)	Females (%)	X <sup>2</sup> (p-value)
ABO BLOOD GROUP			
A	590 (22.09%)	820 (30.68%)	0.001
B	1058 (39.62%)	916 (34.28%)	
O	804 (30.13%)	658 (24.62%)	
AB	218 (8.16%)	278 (10.42%)	
RH BLOOD GROUP			
RH +ve	2558 (95.80%)	2470 (92.44%)	0.000
RH –ve	112 (4.20%)	202 (7.56%)	

### ABO WITH RH BLOOD GROUP

B+ve 35.7% (1906) was the highest frequency ABO blood group in Rh-positive followed by O+ve 25.3% (1352), A+ve 24% (1280) and AB+ve 8.4% (450), while A-ve 35.7% (130) was the highest frequency ABO blood group in Rh negative then O-ve 2.1% (110), B-ve 1.3% (78) and AB-ve 1.0% (46) as shown in table 03.

**TABLE 4: ABO PLUS RH BLOOD GROUP**

ABO plus Rhesus group	FREQUENCY	PERCENT (%)	TOTAL
A +ve	1280	24.0%	5342
B +ve	1906	35.7%	
O+ve	1352	25.3%	
AB +ve	550	8.4%	
A -ve	130	2.4%	
B -ve	78	1.3%	

O -ve	110	2.1%	
AB -ve	46	1.0%	

## DISCUSSION

This research was conducted at a hematology lab within HMC "Hayatabad Medical Complex" in Peshawar over three months. The primary objective was to ascertain the occurrence of "ABO and Rh blood groups" in individuals who sought services at the hematology laboratory of Hayatabad Medical Complex in Peshawar, Pakistan.

The external surface of RBCs (red blood cells) holds antigens that merge with proteins and lipids on the surface of RBC. The "ABO and Rh blood type systems" play a vital function in ensuring safe processes for organ transplants and blood transfusion (15). These blood group antigens are found on the membrane of each "RBC" red blood cell. A blood is considered A if the RBC membrane contains A antigen, while B if the RBC contains B antigen. If the RBC has both antigens, it is considered blood group AB; however, the blood is considered O if no antigen is present on the surface of the RBC(15, 16).

Additionally, reciprocal antibodies are present in the plasma of blood. For instance, the A blood group contains "anti-B" while the B blood group contains "anti-A" in serum/plasma. Likewise, the AB blood group contains no antibodies in the serum/ plasma, while blood group O has both "anti-A and anti-B" in the serum/plasma(15, 16).

Rhesus antigens, including Dd, Cc, and Ee, are also located on the membrane of RBC. Besides these, several other antigens, such as Duffy, Lewis, Kidd, MNS, and Kell, are identified(15). The Rhesus blood group is one of the diverse systems of blood groups comprising almost 45 distinct antigens and, after ABO, which is an integral part of transfusion medicine to be considered (17).

In the current age of information, the wide-ranging geographical variation in ABO blood group genetic expression and the diversity in ABO gene loci have initiated an era of extensive data verification. Due to specific factors, comprehensive comprehension of the geographical and spatial characteristics of blood groups across an entire region or country is rarely achieved(18).

This research study showed B blood group was the most prevalent, followed by "O and A", while the lowest "ABO blood group" was "AB".

In both males and females, the highest frequency was "The ABO blood group was B", and the lowest was "AB"; however, in males, the second most common was O, while in females the second most ABO group was A. In males, the Rh negative was less as compared to Rh negative cases in females; males were 4.20% and females were 7.56%. ABO blood group in Rh positive blood group, the most common was B+ve, followed by O+ve and A+ve, and the least common was AB+ve, however "ABO blood group" in Rh negative, the most common was A-ve followed by O-ve, B-ve while the least common was AB-ve.

Research conducted in Islamabad, Pakistan, by Iqbal, M. et al. observed that among healthy blood donors, the most prevalent blood group was B+ (31.2%), whereas AB- was the least common (0.6%). These results align with the outcomes of our study. They reported the prevalence of other blood groups: A+ (21.5%), AB+ (9.8%), O+ (29.7%), A- (1.8%), B- (2.9%), and O- (2.5%). The proportions of Rhesus positive and Rhesus negative blood groups were 92.2% and 7.8%(10). However, in our investigation, the second most common blood group among Rh-positive individuals was O+, then A+ and AB+. Similarly, the predominant ABO among Rh-negative participants was A-, while the second most common was O-, followed by B- and the least common AB-. Additionally, the frequency of Rh-negative participants in our study was relatively lower than that found in Iqbal, M. et al.'s study(10).

In another study in Dera Ismail Khan, Pakistan, the distribution of blood groups indicated that B+ (33.4%) was the most prevalent, followed by O+ (27.9%), A+ (22.4%), AB+ (7.5%), B- (3.1%), O- (2.7%), A- (2.1%), and AB- (1.0%)(19). These findings were similar to what we discovered in our study. Another study conducted in Lahore, Pakistan, revealed that the B group was the most common frequent (40.45%), then "O (31.06%), A (20.38%), and AB (8.09%)". In terms of the Rhesus (Rh) blood group, 89.48% of individuals were Rhesus (Rh) positive, while nearly 11% were Rh-negative (20). The distribution of ABO blood groups matched our study results, but Rh-negative individuals were slightly high in proportion than in our findings. In a separate study carried out in South India, the descending order of ABO distribution was observed as "O (38%), B (34.5%), A (20.6%), and AB (6.8%)". The proportion of Rhesus

showed 93.4% for D(16). While in this research study, the prevalent ABO blood group was B, a variation that can differ across regions based on genotype prevalence.

A research study into the prevalence of blood groups among Saudis showed O+ showed the highest proportion, then "A+, B+, AB+, O-, A-, B-, and AB-, with exact percentages of 43.8%, 26.28%, 17.66%, 4.32%, 4.14%, 2.02%, 1.62%, and 0.16%", respectively(21). In our research study, B+ was the highest among Rh-positive individuals, while A-ve emerged as the most prevalent among the Rh-negative blood group.

Research conducted in China by Hongmei Liao et al. reported that "O, A, B, and AB blood groups constituted 35.54%, 31.90%, 24.14%, and 8.42%". The Rhesus negative group among blood donors was reported to be 0.55%(22). In our study, B was declared as the highest, and the occurrence of Rh-negative blood types was notably higher compared to the findings of Hongmei Liao et al.'s study(22).

In a study carried out in Algeria, the results indicated that around half of the population exhibited group O (47.52%), then "A (30.14%), group B (16.62%)", and the lowest group AB (5.72%). Additionally, the proportion of Rhesus positive (91.8%) was observed as compared to Rh negative (8.1%) within the population of Algerian (23). Conversely, in our study, the most prevailing ABO blood group was B at 37%, followed by O at 27%, A at 26%, and AB at 10%. The majority, constituting 94%, possessed Rh-positive blood types, while 6% exhibited Rh-negative blood types. The observed phenotypic frequencies for the ABO system were [O] at 46.52%, [B] at 28.80%, [A] at 19.62%, and [AB] at 5.06%. Moreover, the Rh (D) factor was found in 92.72% of [Rh (D) +] and 7.28% of [Rh (D)-ve] (24)

Only those participants were included in this study who reported to the hematology laboratory of HMC (Hayatabad Medical Complex) of Peshawar. The tube method was used for the determination of ABO and Rh blood group by forward and reverse method; however, the Gel card method as a fully automatic method could also be used as a more reliable method.

## CONCLUSION

The highest-frequency ABO among participants was B, and AB was the least found in our study participants. This pattern applied to both genders. Rh-positive types constituted 94%, with Rh-negative at 6%. Among females, Rh-negative was more common than in males. B+ve blood group was

the most prevalent ABO among Rh+ve individuals, while A-ve was the most common among Rh-ve individuals.

**CONFLICT OF INTERESTS:** All authors claim no conflict of interest.

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